



QuikHyb Hybridization Solution

Instruction Manual

Catalog #201220 and #201221

Revision C0

Laboratory Reagent.

201220-12



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QUIKHYB HYBRIDIZATION SOLUTION

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QuikHyb Hybridization Solution

MATERIALS PROVIDED

Material provided	Quantity	
	Catalog #201220	Catalog #201221
QuikHyb hybridization solution ^a	One 250-ml bottle	One 1-liter bottle

^a Store at 4°C. Do not freeze.

Note *The molar concentration of NaCl in QuikHyb hybridization solution is 0.5 M.*

STORAGE CONDITIONS

QuikHyb hybridization solution: 4°C

Revision C0

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INTRODUCTION

Nucleic acid hybridization of a nucleic acid probe to a target sequence immobilized on a solid support such as a nylon membrane is a powerful technique routinely used in the analysis of gene structure and expression and in the diagnosis of disease.

Factors that affect the rate and efficiency of nucleic acid binding to a target sequence include hybridization time, temperature, concentration of the target sequence and concentration of the probe. Other variables which improve the hybridization reaction are the use of rate enhancers and blocking agents to suppress nonspecific hybridization.

QuikHyb hybridization solution was developed for use in Southern, Northern, slot-blot and plaque-lift formats using randomly labeled radioactive and nonradioactive nucleic acid probes. This easy-to-use solution reduces the time required for hybridization from the customary 12–24 hours down to 1–2 hours, resulting in sensitive detection of target sequences.

RADIOACTIVE HYBRIDIZATION WITH QUIKHYB HYBRIDIZATION SOLUTION

Prehybridization

Notes *Gently invert the QuikHyb hybridization solution immediately before use in order to ensure a homogenous mixture.*

Prior to prehybridization (after crosslinking or baking), briefly dip the membrane in deionized water to remove excess salt from the membrane.

The membrane should be covered with a thin film of the QuikHyb solution at all times.

1. Use a minimum of 33 μl of the QuikHyb hybridization solution/cm² of the blot. If this volume is not sufficient to cover the membrane at all times during the prehybridization and hybridization, add more solution until it does.

Minimum Total Volume

2 ml when using 50-ml conical tubes
10 ml when using heat-sealable bags or roller bottles

2. For *double-stranded probes*, prehybridize the membrane in QuikHyb solution at 68°C for 10–20 minutes.

For *oligonucleotide probes and riboprobes*, calculate the melting temperature (T_m) (see *Appendix I: Hybridization and Melting Temperatures* for the mathematical formula). Prehybridize the membrane in QuikHyb solution for 10–20 minutes at 5–10°C below the T_m .

Probe Preparation and Hybridization

Add the labeled probe to 100 μ l of 10 mg/ml sonicated salmon sperm DNA.

Suggested Probe Concentration

2.5×10^6 total counts/2 ml of hybridization solution

Specific Activity of the Probe

10^8 cpm/ μ g or greater

Double-Stranded Probes

Boil the double-stranded probe and salmon sperm DNA mixture for 2 minutes and then add the mixture to the prehybridization solution. Due to the viscosity of the QuikHyb hybridization solution, the probe may not mix well in the large glass tubes (roller bottles) used in hybridization ovens. Perform the following steps to ensure the probe is mixed well with the prehybridization solution.

1. Remove 1 ml of the prehybridization solution from the glass tube and mix the solution with the probe.
2. Place this mixture back in the glass tube and hybridize in QuikHyb solution at 68°C for 1 hour.

Oligonucleotide Probes and Riboprobes

Add the probe and salmon sperm DNA mixture directly to the prehybridization solution without boiling. Due to the viscosity of the QuikHyb hybridization solution, the probe may not mix well in the large glass tubes (roller bottles) used in hybridization ovens. Perform the following steps to ensure the probe is mixed well with the prehybridization solution.

1. Remove 1 ml of the prehybridization solution from the glass tube and mix the solution with the probe.
2. Place this mixture back in the glass tube and hybridize in QuikHyb solution at 5–10°C below the T_m for 1 hour (see *Appendix I: Hybridization and Melting Temperatures*).

Washing the Membrane

Perform the following washes (gentle agitation is required) for the double-stranded probes, oligonucleotide probes and riboprobes:

1. Wash twice for 15 minutes at room temperature with a 2× SSC buffer and 0.1% (w/v) SDS wash solution[§] (~2.5 ml/cm²).
2. Wash once for 30 minutes at 60°C with a 0.1× SSC buffer and 0.1% (w/v) SDS wash solution[§] for a high-stringency wash.

Detection

Wrap the membrane in plastic wrap and place the wrapped membrane on Kodak X-OMAT AR film with an intensifying screen at –80°C. Expose the film overnight.

Stripping the Membrane for Reuse

1. Heat the 0.1× SSC buffer and 0.1% (w/v) SDS wash solution to boiling.
2. In a glass dish, pour the 0.1× SSC buffer and 0.1% (w/v) SDS wash solution over the membrane and wash the membrane twice for 15 minutes.
3. Proceed with the prehybridization step for the next hybridization or store the blot in plastic wrap, desiccated.

[§] See *Preparation of Media and Reagents*.

APPENDIX I: HYBRIDIZATION AND MELTING TEMPERATURES

Calculating the Hybridization Temperature

The hybridization temperature for oligonucleotide probes equals 5–10°C below the T_m . For best results with oligonucleotide probes, perform hybridizations for 1 hour.

Calculating the Melting Temperature¹

Notes *The first method below overestimates the T_m of hybrids involving longer nucleotides. The second formula works only for Na^+ concentrations of $\leq 1 M$.*

The molar concentration of NaCl in QuikHyb hybridization solution is 0.5 M.

Oligonucleotides Shorter than 18 Bases

$$T_m = 2^\circ\text{C}(\text{A} + \text{T}) + 4^\circ\text{C}(\text{G} + \text{C})$$

Oligonucleotides 14 Bases and Longer (up to 60–70 Nucleotides)

$$T_m = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G + C) - (600/N)$$

where N is the chain length.

PREPARATION OF MEDIA AND REAGENTS

20× SSC Stock Solution 175.3 g of NaCl (3 M final concentration) 88.2 g of sodium citrate-trisodium salt (300 mM final concentration) Adjust the pH to 7.0 with HCl Add distilled water (dH ₂ O) to 1 liter	20% (w/v) SDS Stock Solution Dissolve 20 g of SDS in 90 ml of distilled water Mix well and heat to 68°C if necessary Then add dH ₂ O to a final volume of 100 ml
2× SSC Buffer and 0.1% (w/v) SDS Wash Solution 100 ml of 20× SSC buffer 5 ml of 20% (w/v) SDS dH ₂ O to 1 liter	0.1× SSC Buffer and 0.1% (w/v) SDS Wash Solution 5 ml of 20× SSC buffer 5 ml of 20% (w/v) SDS dH ₂ O to 1 liter

REFERENCE

1. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at www.agilent.com. MSDS documents are not included with product shipments.

QuikHyb Hybridization Solution

Catalog #201220 (250 ml) and #201221 (1 liter)

QUICK-REFERENCE PROTOCOL

Radioactive Hybridization

- ◆ Prehybridize for 15 minutes
- ◆ Denature the double-stranded probe
- ◆ Add probe to the prehybridizing blot
- ◆ Hybridize for 1 hour
- ◆ Wash twice for 15 minutes at room temperature with a $2\times$ SSC buffer and 0.1% (w/v) SDS wash solution
- ◆ Wash once for 30 minutes at higher stringency temperature with a $0.1\times$ SSC buffer and 0.1% (w/v) SDS wash solution
- ◆ Expose to Kodak X-OMAT AR film at -80°C overnight